

Amendments to the Claims

Claims 1. (Withdrawn) A method, comprising:

- a) providing a tRNA and a BODIPY marker; and
- b) aminoacylating said tRNA molecule with said BODIPY marker to create a misaminoacylated tRNA.

Claim 2. (Withdrawn) The method of claim 1, further comprising:

- c) introducing said misaminoacylated tRNA into a translation system under conditions such that said marker is incorporated into a nascent protein.

Claim 3. (Withdrawn) The method of claim 2, further comprising:

- d) detecting said nascent protein containing said marker.

Claim 4. (Withdrawn) The method of claim 3, further comprising:

- e) isolating said detected nascent protein.

Claim 5. (Withdrawn) The method of claim 3, wherein the nascent protein detected is selected from recombinant gene products, gene fusion products, enzymes, cytokines, carbohydrate and lipid binding proteins, nucleic acid binding proteins, hormones, immunogenic proteins, human proteins, viral proteins, bacterial proteins, parasitic proteins and fragments and combinations thereof.

Claim 6. (Withdrawn) The method of claim 2, wherein the translation system comprises a cellular or cell-free translation system.

Claim 7. (Withdrawn) The method of claim 5, wherein the translation system is selected from the group consisting of tissue culture cells, primary cells, cells in vivo, isolated immortalized cells, human cells and combinations thereof.

Claim 8. (Withdrawn) The method of claim 5, wherein the cell-free translation system is

selected from the group consisting of Escherichia coli lysates, wheat germ extracts, insect cell lysates, rabbit reticulocyte lysates, frog oocyte lysates, dog pancreatic lysates, human cell lysates, mixtures of purified or semi-purified translation factors and combinations thereof.

Claim 9. (Withdrawn) The method of claim 5, wherein said cell-free translation system is incubated at a temperature of between about 25°C to about 45°C.

Claim 10. (Withdrawn) The method of claim 5, wherein the cell-free translation system is a continuous flow or dialysis system.

Claim 11. (Withdrawn) The method of claim 1, wherein the tRNA molecule is misaminoacylated by chemical or enzymatic means.

Claim 12. (Withdrawn) The method of Claim 2, wherein two or more different misaminoacylated tRNAs are introduced into the translational system.

Claim 13. (Withdrawn) The method of claim 3, wherein said nascent protein detected is functionally active.

Claim 14 (Withdrawn) The method of claim 1, wherein said tRNA molecule is an initiator tRNA molecule.

Claim 15. (Withdrawn) The method of claim 1, wherein said tRNA molecule is a suppressor tRNA molecule.

Claim 16 (Withdrawn). A kit, comprising:

- a) a first containing means containing at least one component of a protein synthesis system;
- b) a second containing means containing a misaminoacylated tRNA, wherein said marker of said tRNA is misaminoacylated with a BODIPY marker.

Claim 17. (Withdrawn) The kit of claim 16, wherein said misaminoacylated tRNA comprises an initiator tRNA.

Claim 18. (Withdrawn) The kit of claim 16, wherein said misaminoacylated tRNA comprises a suppressor tRNA.

Claim 19. (Withdrawn) The kit of claim 16, wherein said component of said protein synthesis system comprises ribosomes.

Claim 20. (Withdrawn) A tRNA molecule misaminoacylated with a BODIPY marker.

Claim 21. (Currently Amended) A gel-free method, comprising:

a) providing i) a misaminoacylated initiator tRNA molecule which only recognizes the first AUG codon that serves to initiate protein synthesis, said misaminoacylated initiator tRNA molecule comprising a first marker, and ii) a nucleic acid template encoding a protein, said protein comprising an affinity marker and a C-terminal marker; ~~and~~

b) introducing said misaminoacylated initiator tRNA to a translation system comprising said template under conditions such that a nascent protein is generated, said protein comprising said first marker, said affinity marker, and said C-terminal marker; and

c) testing said nascent protein under gel-free conditions that permit detection of a truncated protein.

Claim 22. (Original) The method of claim 21, wherein the nascent protein generated is selected from recombinant gene products, gene fusion products, enzymes, cytokines, carbohydrate and lipid binding proteins, nucleic acid binding proteins, hormones, immunogenic proteins, truncated proteins, mutant proteins, human proteins, viral proteins, bacterial proteins, parasitic proteins and fragments and combinations thereof.

Claim 23. (Original) The method of claim 21, wherein the translation system comprises a cellular or cell-free translation system.

Claim 24. (Original) The method of claim 23, wherein the cellular translation system is selected from prokaryotic cells, eukaryotic cells, tissue culture cells, primary cells, cells in vivo, isolated immortalized cells, human cells and combinations thereof.

Claim 25. (Original) The method of claim 23, wherein the cell-free translation system is selected from the group consisting of Escherichia coli lysates, wheat germ extracts, insect cell lysates, rabbit reticulocyte lysates, frog oocyte lysates, dog pancreatic lysates, human cell lysates, mixtures of purified or semi-purified translation factors and combinations thereof.

Claim 26. (Original) The method of claim 23, wherein said cell-free translation system is incubated at a temperature of between about 25°C to about 45°C.

Claim 27. (Original) The method of claim 23, wherein the cell-free translation system is a continuous flow or dialysis system.

Claim 28. (Original) The method of claim 21, wherein the tRNA molecule is aminoacylated by chemical or enzymatic misaminoacylation.

Claim 29. (Original) The method of claim 23, wherein said nascent protein is functionally active.

Claim 30. (Original) The method of claim 21, wherein said first marker comprises a fluorescent compound.

Claim 31. (Original) The method of claim 21, wherein said fluorescent compound is selected from dipyrrometheneboron difluoride (4,4-difluoro-4-bora-3a,4a-diaza-s-indacene) dyes and derivatized coumarin.

Claim 32. (Original) The method of claim 21, wherein said C-terminal comprises a histidine tag.

Claim 33. (Currently Amended) A gel-free method, comprising:

a) providing i) a misaminoacylated initiator tRNA molecule which only recognizes the first AUG codon that serves to initiate protein synthesis, said misaminoacylated initiator tRNA molecule comprising a first marker, and ii) a nucleic acid template encoding a protein, said protein comprising an affinity marker and a C-terminal marker; ~~and~~

b) introducing said misaminoacylated initiator tRNA to a translation system comprising said template under conditions such that a nascent protein is generated, said protein comprising said first marker at the N-terminus of said protein, a said C-terminal marker, and said affinity marker adjacent to said first marker-; and

c) testing said nascent protein under gel-free conditions that permit detection of a truncated protein.

Claim 34. (Original) The method of claim 33, wherein the nascent protein generated is selected from recombinant gene products, gene fusion products, enzymes, cytokines, carbohydrate and lipid binding proteins, nucleic acid binding proteins, hormones, immunogenic proteins, truncated proteins, mutant proteins, human proteins, viral proteins, bacterial proteins, parasitic proteins and fragments and combinations thereof.

Claim 35. (Original) The method of claim 33, wherein the translation system comprises a cellular or cell-free translation system.

Claim 36. (Original) The method of claim 35, wherein the cellular translation system is selected from prokaryotic cells, eukaryotic cells, tissue culture cells, primary cells, cells in vivo, isolated immortalized cells, human cells and combinations thereof.

Claim 37. (Original) The method of claim 35, wherein the cell-free translation system is

selected from the group consisting of Escherichia coli lysates, wheat germ extracts, insect cell lysates, rabbit reticulocyte lysates, frog oocyte lysates, dog pancreatic lysates, human cell lysates, mixtures of purified or semi-purified translation factors and combinations thereof.

Claim 38. (Original) The method of claim 35, wherein said cell-free translation system is incubated at a temperature of between about 25°C to about 45°C.

Claim 39. (Original) The method of claim 35, wherein the cell-free translation system is a continuous flow or dialysis system.

Claim 40. (Original) The method of claim 33, wherein the tRNA molecule is aminoacylated by chemical or enzymatic misaminoacylation.

Claim 41. (Original) The method of claim 33, wherein said nascent protein is functionally active.

Claim 42. (Original) The method of claim 33, wherein said first marker comprises a fluorescent compound.

Claim 43. (Original) The method of claim 42, wherein said fluorescent compound is selected from dipyrrometheneboron difluoride (4,4-difluoro-4-bora-3a,4a-diaza-s-indacene) dyes and derivatized coumarin.

Claim 44. (Original) The method of claim 13, wherein said C-terminal comprises a histidine tag.

Claim 45. (Currently Amended) A gel-free method, comprising:

a) providing i) a misaminoacylated tRNA molecule which only recognizes the first codon designed to serve to initiate protein synthesis, said misaminoacylated initiator

tRNA molecule comprising a first marker, and ii) a nucleic acid template encoding a protein, said protein comprising an affinity marker and a C-terminal marker; ~~and~~

b) introducing said misaminoacylated initiator tRNA to a translation system comprising said template under conditions such that a nascent protein is generated, said protein comprising said first marker, said affinity marker and said C-terminal marker;

and

c) testing said nascent protein under gel-free conditions that permit detection of a truncated protein.

Claim 46. (Original) The method of claim 45, wherein the nascent protein generated is selected from recombinant gene products, gene fusion products, enzymes, cytokines, carbohydrate and lipid binding proteins, nucleic acid binding proteins, hormones, immunogenic proteins, truncated proteins, mutant proteins, human proteins, viral proteins, bacterial proteins, parasitic proteins and fragments and combinations thereof.

Claim 47. (Original) The method of claim 45, wherein the translation system comprises a cellular or cell-free translation system.

Claim 48. (Original) The method of claim 47, wherein the cellular translation system is selected from prokaryotic cells, eukaryotic cells, tissue culture cells, primary cells, cells in vivo, isolated immortalized cells, human cells and combinations thereof.

Claim 49. (Original) The method of claim 47, wherein the cell-free translation system is selected from the group consisting of Escherichia coli lysates, wheat germ extracts, insect cell lysates, rabbit reticulocyte lysates, frog oocyte lysates, dog pancreatic lysates, human cell lysates, mixtures of purified or semi-purified translation factors and combinations thereof.

Claim 50. (Original) The method of claim 47, wherein said cell-free translation system is incubated at a temperature of between about 25°C to about 45°C.

Claim 51. (Original) The method of claim 47, wherein the cell-free translation system is a continuous flow or dialysis system.

Claim 52. (Original) The method of claim 45, wherein the tRNA molecule is aminoacylated by chemical or enzymatic misaminoacylation.

Claim 53. (Original) The method of claim 45, wherein said nascent protein is functionally active.

Claim 54. (Original) The method of claim 45, wherein said first marker comprises a fluorescent compound.

Claim 55. (Original) The method of claim 44, wherein said fluorescent compound is selected from dipyrrometheneboron difluoride (4,4-difluoro-4-bora-3a,4a-diaza-s-indacene) dyes and derivatized coumarin.

Claim 56. (Original) The method of claim 45, wherein said C-terminal comprises a histidine tag.

Claim 57. (Currently Amended) A gel-free method, comprising:

a) providing i) a misaminoacylated tRNA molecule which only recognizes the first codon designed to serve to initiate protein synthesis, said misaminoacylated initiator tRNA molecule comprising a first marker, and ii) a nucleic acid template encoding a protein, said protein comprising an affinity marker and a C-terminal marker; ~~and~~

b) introducing said misaminoacylated initiator tRNA to a translation system comprising said template under conditions such that a nascent protein is generated, said protein comprising said first marker at the N-terminus of said protein, a C-terminal marker, and said affinity marker adjacent to said first marker; and

c) testing said nascent protein under gel-free conditions that permit detection of a truncated protein.

Claim 58. (Original) The method of claim 57, wherein the nascent protein generated is selected from recombinant gene products, gene fusion products, enzymes, cytokines, carbohydrate and lipid binding proteins, nucleic acid binding proteins, hormones, immunogenic proteins, truncated proteins, mutant proteins, human proteins, viral proteins, bacterial proteins, parasitic proteins and fragments and combinations thereof.

Claim 59. (Original) The method of claim 58, wherein the translation system comprises a cellular or cell-free translation system.

Claim 60. (Original) The method of claim 59, wherein the cellular translation system is selected from prokaryotic cells, eukaryotic cells, tissue culture cells, primary cells, cells in vivo, isolated immortalized cells, human cells and combinations thereof.

Claim 61. (Original) The method of claim 59, wherein the cell-free translation system is selected from the group consisting of Escherichia coli lysates, wheat germ extracts, insect cell lysates, rabbit reticulocyte lysates, frog oocyte lysates, dog pancreatic lysates, human cell lysates, mixtures of purified or semi-purified translation factors and combinations thereof.

Claim 62. (Original) The method of claim 59, wherein said cell-free translation system is incubated at a temperature of between about 25°C to about 45°C.

Claim 63. (Original) The method of claim 59, wherein the cell-free translation system is a continuous flow or dialysis system.

Claim 64. (Original) The method of claim 57, wherein the tRNA molecule is aminoacylated by chemical or enzymatic misaminoacylation.

Claim 65. (Original). The method of claim 57, wherein said nascent protein is functionally active.

Claim 66. (Original) The method of claim 57, wherein said first marker comprises a fluorescent compound.

Claim 67. (Original) The method of claim 66, wherein said fluorescent compound is selected from dipyrrometheneboron difluoride (4,4-difluoro-4-bora-3a,4a-diaza-s-indacene) dyes and derivatized coumarin.

Claim 68. (Original) The method of claim 57, wherein said C-terminal comprises a histidine tag.